

Review of King et al. (pre-publication copy): Comprehensive analysis of molecular phylogeographic structure among the meadow jumping mice (*Zapus hudsonius*) reveals evolutionarily distinct subspecies.

1. Techniques, methods, and markers.—King et al. employed 21 microsatellite loci to assay genetic variation in the nuclear genome; as well as 374 bps of the control region and 906 bps from the cytochrome b gene to assay genetic variation in the mitochondrial genome across populations within and among several subspecies of *Z. hudsonius*, with a focus on the level of differentiation and evolutionary distinctness of the subspecies *Z. h. preblei*. The number of microsatellite loci employed is impressive and, in using well-justified analytical techniques, provides a robust estimate of the geographic structure of genetic variation across sampled populations. The approaches used for mtDNA analysis are justified in accord with the relatively shallow levels of genetic diversity across the sampled populations. The total number of populations sampled is fairly small, but the geographic range sampled is sufficient to encompass overall phylogeographic structure of genetic variation with a focus on *Z. h. preblei*; the samples sizes per population, however, are very large and ensure that within-population genetic variation has been captured to an impressive degree.
2. Taxonomic validity of *Z. h. preblei* and neighboring subspecies.—Given the sampling design—with an explicit focus on *Z. h. preblei* and sufficient sampling in surrounding subspecies to address that focal issue—and given caveats associated with the range of opinions and definitions across evolutionary biologists regarding the conceptual and diagnostic validity of the subspecies-level taxonomic category, I support the author's conclusions that *Z. h. preblei* has sufficient evolutionary distinctness to be recognizable as a valid subspecies. I believe that more thorough geographic sampling throughout the remaining range of the species, using the same techniques and markers employed here, would be necessary to address the validity of neighboring subspecies.
3. Should *Z. h. preblei* be managed as two distinct population segments?—I think there is sufficient evidence from the microsatellite analyses that there is some differentiation among sets of populations within *Z. h. preblei*, and it is most likely associated with a north vs. south geographic gradient. If the most relevant management question is whether individuals from either of these sets of populations should be translocated into the other, some caution is probably warranted because these data provide evidence for a number of generations of population-level isolation, and as such, perhaps development of local adaptations (although these results provide no direct evidence that the latter has occurred).
4. Possible alternative explanations.—I believe that the strength of this study in demonstrating evolutionary distinctness of *Z. h. preblei* comes from the thorough sampling of variation across both nuclear and mitochondrial genomes, with both showing congruent evidence of distinctness. Such congruence would be much less likely if, for example, geographic pattern resulted from much greater female than

male philopatry (in which case mtDNA would show more structure than nucDNA), or perhaps local adaptation (in which case there should not be general agreement across a wide range of unlinked microsatellite loci). The overall signal of a geographic pattern (two distinct groups of “subspecies”—*Z. h. preblei* with a northern plains affinity; apart from a central and southern group) indicates a strong historical biogeographic contribution to current geographic genetic structure in the species and sheds light on the origins of *Z. h. preblei*.

5. Additional analyses needed to verify assertions.—I think the major assertion at issue—the evolutionary distinctness of *Z. h. preblei*—is supported robustly in this study. It would be nice to see the geography of mitochondrial and nuclear variation filled in at some point. For example, populations along the front range (north into Wyoming, south into New Mexico) could be sampled with the goal of delineating more precisely the overall distribution of diagnosable *Z. h. preblei* genotypes.
6. Conflicting conclusions.—King et al. (lines 637-651) emphasize the strengths of their sampling strategy for finding statistical differences between previously delineated subspecies. However, if one is trying to delineate with precision the geographic extent of evolutionary lineages (whether one calls them subspecies or not) the sampling strategy of Ramey et al. would be better and still has an important place in future phylogeographic studies of this species. We have both ends of the spectrum here now—many individuals in a few representative populations vs. a few individuals across many more populations. It would be nice eventually to have a data set from the latter that has the amount of molecular data employed in this study. King et al. provide a convincing criticism of the dangers involved with use of museum skins for DNA and the sorts of controls that should be employed to avoid contamination and variation among sequences due to preservation artifacts. Apart from (or more accurately, driving) different sampling designs and analytical techniques, the conflicting conclusions result from different perspectives on evidence needed to recognize evolutionary divergence that is of sufficient distinctness to support taxonomic recognition at a subspecies rank and/or recognition of discrete population segments worthy of legal consideration. This continues to be a big debate among evolutionary and conservation biologists, but regardless of individual opinion on significance, King et al. provide a convincing case for the evolutionary distinctness of *Z. h. preblei*.